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Original Article

Article title: Increasing stomatal conductance in response to rising atmospheric CO_2

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Running title: Stomatal conductance and elevated CO_2

ABSTRACT

Background and Aims: Studies have indicated that plant stomatal conductance (g_s) decreases in response to elevated atmospheric CO_2, a phenomenon of significance for the global hydrological cycle. However, g_s increases across certain CO_2 ranges have been predicted by optimisation models. The aim of this work was to demonstrate that under certain environmental condition, g_s can increase in response to elevated CO_2.

Methods: When using (i) an extensive, up-to-date, synthesis of g_s responses in FACE experiments, (ii) *in situ* measurements across four biomes showing dynamic g_s responses to a CO_2 rise of ~50ppm (characterising the change in this greenhouse gas over the past three decades) and (iii) a photosynthesis-stomatal conductance model, it is demonstrated that g_s can in some cases *increase* in response to increasing atmospheric CO_2.

Key Results: Field observations are corroborated by an extensive synthesis of g_s responses in FACE experiments showing that 11.8% of g_s responses under experimentally elevated CO_2 are positive. They are further supported by a strong data-model fit (r^2=0.607) using a stomatal optimization model applied to the field g_s dataset. A parameter space identified in the Farquhar-Ball-Berry photosynthesis-stomatal conductance model confirms field observations of increasing g_s under elevated CO_2 in hot dry conditions. It was shown that contrary to the general assumption, positive g_s responses to elevated CO_2, although relatively rare, are a feature of woody taxa adapted to warm, low-humidity conditions, and that this response is also demonstrated in global simulations using the Community Land Model (CLM4).

Conclusions: The results contradict the over-simplistic notion that global vegetation always responds with decreasing g_s to elevated CO_2, a finding that has important implications for predicting future vegetation feedbacks on the hydrological cycle at the regional level.

Key words: Stomata, stomatal conductance, climate change, CO_2, hydrology, CLM,

vegetation, run-off, drought, photosynthesis, temperature, VPD

INTRODUCTION

Water loss through plant stomata- small pores on the surface of leaves through which gas exchange between plants and the atmosphere takes place - is an unavoidable trade-off in the exchange for CO_2, the substrate for photosynthesis. Decreased stomatal conductance (g_s), via physiological (stomata responding dynamically to environmental stimuli) and/or morphological changes (via alteration in stomatal density and size) has been observed in elevated carbon dioxide (CO_2) environments in both laboratory and Free Air CO_2 Enrichment (FACE) studies (Ainsworth and Rogers 2007; Drake *et al.* 1997; Farquhar and Sharkey 1982; Leuzinger and Körner 2007; Woodward 1987). However, recent studies suggest that rising atmospheric CO_2-induced decreases in g_s may be offset by contemporaneous increases of leaf area index (LAI) during the course of a growing season (Frank *et al.* 2015; Niu *et al.* 2013; Piao *et al.* 2007; Schymanski *et al.* 2015; Wu *et al.* 2012). Thus, despite significant improvements in our understanding of plant-atmosphere interactions in recent years, the net stomatal conductance response of the entire global vegetation system to rising anthropogenic CO_2 remains unclear.

In addition, little is known regarding the physiological response of plants to increasing CO_2 across multiple biomes, and in varying temperature and humidity regimes. For example, FACE studies are predominantly limited to the mid-latitudes of the northern hemisphere (Fig. 1), biasing our understanding of plant responses to these regions. Moreover, disparate vegetation responses in dry and drought prone environments have been reported (Choat *et al.* 2012; De Kauwe *et al.* 2015; Limousin *et al.* 2013; Mencuccini *et al.* 2015; Zhou *et al.* 2013). It is therefore critical to improve our understanding of these responses in order to better predict future freshwater cycling, especially in regions vulnerable to drought and desertification in the 21st century (Lawrence *et al.* 2011).

Here we demonstrate that g_s can in some cases *increase* in response to increasing atmospheric CO_2. This is shown using (i) *in situ* measurements of 51 woody plant taxa across four biomes showing dynamic g_s responses to a CO_2 rise of ~50ppm which represents the change in this greenhouse gas over the past three decades, (ii) an extensive, up-to-date, synthesis of g_s responses in FACE experiments, (iii) both the stand-alone and Community Land Model version 4 (CLM4)-integrated application of the Farquhar-Ball-Berry photosynthesis-stomatal conductance model and (iv) the Medlyn *et al.* (2011) optimal stomatal model.

MATERIALS AND METHODS

Synthesis of Free Air CO_2 Enrichment (FACE) studies

A literature review was undertaken of studies that specifically focused on the effect of elevated CO_2 on plant stomatal conductance (g_s) in FACE experiments. A total of 51 studies were included in the database (Adachi *et al.* 2014; Ainsworth and Rogers 2007; Ainsworth *et al.* 2003; Bader *et al.* 2010; Bhattacharya *et al.* 1994; Borjigidai *et al.* 2006; Bryant *et al.* 1998; Calfapietra *et al.* 2005; Chen *et al.* 2014; Ellsworth 1999; Ellsworth *et al.* 1995; Ellsworth *et al.* 2012; Garcia *et al.* 1998; Ghini *et al.* 2015; Grant *et al.* 1999; Gunderson *et al.* 2002; Hamerlynck *et al.* 2002; Hamerlynck *et al.* 2000; Hao *et al.* 2013; Hättenschwiler *et al.* 2002; Herrick *et al.* 2004; Herrick and Thomas 1999; Herrick and Thomas 2003; Hileman *et al.* 1994; Hileman *et al.* 1992; Huxman and Smith 2001; Ji *et al.* 2015; Keel *et al.* 2006; Leakey *et al.* 2006; Lee *et al.* 2001; Marchi *et al.* 2004; McElrone *et al.* 2005; Naumburg and Ellsworth 2000; Naumburg *et al.* 2003; Naumburg *et al.* 2004; Neal *et al.* 2000; Nijs *et al.* 1997; Noormets *et al.* 2001; Nowak *et al.* 2001; Pataki *et al.* 2000; Pearson *et al.* 1995;

Rogers *et al.* 2004; Ruhil *et al.* 2015; Shimono *et al.* 2010; Singsaas *et al.* 2000; Tricker *et al.* 2005; Wall *et al.* 2000; Wall *et al.* 2001; Wechsung *et al.* 2000; Wullschleger *et al.* 2002; Yoshimoto *et al.* 2005). The FACE synthesis was built on the original data set by Ainsworth and Rogers (2007). Values reported in tables and in the text were taken directly from publications, whereas results in graphs were digitized. Individual independent observations were obtained following the longest period of CO_2 exposure reported in each study (independent = plant; repeated = species). Studies that examined multi-factorial designs could have contributed several observations for each response variable (e.g. drought, nitrogen enrichment etc.). The mean, standard deviation (SD) and the effect size of the treatment (Ne) and relative control treatment (Na) were recorded. If standard error (SE) was reported we transformed these according to SE=SD*[(n-1)/2]. Database records typically included the year and month the data were collected, GPS site locations, ambient CO_2, elevated CO_2, study organism (incl. varieties), plant functional type (PFT), photosynthetic pathway and other experimental treatments (e.g. nitrogen fertilization). Stomatal conductance measurements from 52 different species, within seven PFTs (C3 crops, C3 forbs, C3 grasses, C3 herbs, C3 shrubs and C3 conifer and C3 broadleaved trees) were included in the analysis. The ranges of ambient and elevated CO_2 between studies were 350-411ppm and 538-680ppm respectively. A kernel density estimation was used to visualise the stomatal conductance data by estimating the unknown probability of the data, based on a sample of points taken from that distribution.

Dynamic g_s responses to CO_2 change (across four biomes)

Assessment of the dynamic stomatal responses to increasing CO_2 across four different biomes (including a tropical seasonal biome which had been subjected to drought) was achieved during a 10-week scientific expedition to North and Central America in the summer of 2014.

A total of 51 woody tree and shrub species were measured with a CIRAS-2 gas analyser (PP-Systems, Amesbury, MA, USA) attached to a PLC6 (U) cuvette fitted with a 1.7 cm^2 measurement window and a red/white light LED unit.

Measurements were carried out (Fig. 3) at two boreal forest sites (16 species, Bird Creek [60°58'N, 149°28'W] and Kenai [60°33.3'N, 151°12.8'W], Alaska, USA), one temperate deciduous forest site (11 species, Smithsonian Environmental Research Centre [38°53'N, 76°32'W], Maryland, USA), two tropical seasonal forest (wet) sites (15 species, Cambalache [18°27'N, 66°35'W] and Guajataca [18°24'N, 66°58'W], Puerto Rico) one of which had undergone a long drought period (Cambalache), and one tropical seasonal forest (dry) site (9 species, Guanica [17°93'N, 66°92'W], Puerto Rico). See Table S1 for a complete species list.

Stomatal responses were assessed on an average of four individuals per species between 9:00 am and 13:00 pm. A sun exposed branch was sampled following standard protocol (Berveiller *et al.* 2007; Dang *et al.* 1997; Domingues *et al.* 2010; Koch *et al.* 2004; Rowland *et al.* 2015) from each individual using either a pruner (shrubs) or a pole with a scythe fitted on its top (trees) and was immediately recut under water. Following this, a fully expanded leaf from each branch was enclosed in the cuvette of the gas analyser, which was running at a sub-ambient ~year 1990 reference CO_2 concentration of 354ppm (Betts *et al.* 2016). Stomatal conductance at sub-ambient CO_2 concentration was recorded upon stabilisation of its value, which typically took less than 15 minutes. Subsequently, reference CO_2 was established at 400ppm (year 2016 values) (Betts *et al.* 2016) and the leaf was left to equilibrate for at least 15 minutes before g_s at modern ambient CO_2 was recorded. Randomization of the sequence of the two treatments was ensured; overall about 65% of the measurements started at 400ppm (386.6±0.5) and were reduced to 354ppm (342.4±0.5), while the rest of measurements (35%)

started at 354 ppm and were increased to 400ppm. On several occasions the reversibility of the CO_2 effects on g_s was tested. This was done by measuring g_s at a starting CO_2 concentration of 400ppm, after which CO_2 was reduced to 354ppm for several minutes, before it was returned to the initial concentration of 400ppm. The final g_s values at 400ppm were the same as those initially recorded (data not shown).

Stomatal responses to a subtle increase in CO_2 were estimated as the percentage change in the g_s values between sub-ambient CO_2 and modern ambient CO_2. Air flow, light intensity and incoming mole fraction of water during the measurements were maintained at 200 cm^3 min^{-1}, 1000 $\mu molm^{-2}s^{-1}$ and 80-90 % of ambient respectively. Since ambient and leaf temperatures varied significantly between the beginning and the end of the daily measurement time window in all biomes, the measurements were taken at the calculated mean and biome-specific leaf temperature at 9:00 am. Calculation was performed early on the first measurement day at each site by running the gas analyser at the set points mentioned above (i.e. 1000 $\mu molm^{-2}s^{-1}$ of light, 80-90 % of ambient water vapour, 400 $\mu molmol^{-1}$ CO_2, no temperature control) and by recording the leaf temperatures of at least 10 leaves belonging to 10 different species growing at the site. Differences in g_s responses between biomes were tested on the normal data using ANOVA analysis. Moreover, a linear model was used to test for the correlation of g_s to VPD and leaf temperature and the modelled and observed g_s data. Mixed effects models were used to test which variables best explain the observed changes in g_s and the best model was selected following Akaike Information Criterion (AIC).

Farquhar-Ball-Berry model (combined photosynthesis and g_s)

The model relates g_s to net leaf photosynthesis, scaled by the relative humidity at the leaf surface and the CO_2 concentration at the leaf surface (Collatz *et al.* 1991; Sellers *et al.* 1996). It solves the following three equations:

$$g_s = mA \frac{e_a}{e_i} \frac{p_a}{c_a} + b \qquad (1)$$

$$A = \frac{g_s}{1.65} \frac{(c_a - c_i)}{p_a} \qquad (2)$$

$$A = \min(w_c; w_j; w_e) \qquad (3)$$

where g_s is the stomatal conductance to water vapour, A is the photosynthetic uptake flux of CO_2, c_a and c_i are partial pressures of CO_2 just outside and inside the stomata respectively, p_a=10^5 Pa is atmospheric pressure, e_a and e_i the water vapour pressures just outside and inside the stomata respectively (the latter computed as the saturation vapour pressure at the leaf temperature T_v), and m and b are empirical constants taken as m = 6 and b = 3x10^4 μmol m^{-2} s^{-1}. The uptake flux is taken to be the minimum of three rate-limiting processes for C_3 plants: Rubisco limitation, $w_c = V_{cmax} (c_i - \Gamma^*) / (c_i + K_c + o_i K_c / K_o)$; light limitation, $w_j = \alpha$ PAR $(c_i - \Gamma^*) / (c_i + 2\Gamma^*)$, and export limitation $w_e = 0.5\, V_{cmax}$. In these expressions K_c and K_o are Michaelis-Menten constants for CO_2 and O_2 respectively which vary with leaf temperature T_v (expressed in ^{o}C) as $K_c = K_{c25} a_{kc}^{(T_v-25)/10}$ and $K_o = K_{o25} a_{ko}^{(T_v-25)/10}$ where K_{c25} = 30 and K_{o25} = 30000 are reference values while a_{kc} = 2.1 and a_{ko} =1.2. The CO_2 compensation point is taken as $\Gamma^* = 0.105\, o_i K_c / K_o$ with o_i the partial pressure of oxygen. PAR=1000 μmol m^{-2} s^{-1} is the photosynthetically active radiation flux falling on the leaf, and α=0.06 is the quantum efficiency of photosynthesis. Finally, V_{cmax} is the temperature-dependent maximum carboxylation rate modelled following Katul *et al.* (2009) as $V_{cmax} = V_{cmax25}\, e^{0.88(Tv-25)} / (1 + e^{0.29(Tv-41)})$ where V_{cmax25} = 60 μmol m^{-2} s^{-1} is the maximum carboxylation rate at 25^{o}C. Given values of c_a, e_a, T_v, PAR and V_{cmax25}, the equations are solved numerically using an iterative method to yield c_i, A and g_s.

Optimisation model

For the comparison of our field data with the optimum g_s model of Medlyn *et al.* (2011) we used measured values of A, c_a and VPD and PFT specific g_i values for evergreen and deciduous species from Lin *et al.* (2015). We assumed that g_0 was 20 mmol m^{-2} s^{-1}. The optimal model was as follows:

$$g_s = g_0 + \left(1 + \frac{g_1}{\sqrt{D}}\right)\frac{A}{c_a} \qquad (4)$$

where D is VPD (kPa), g_l is the model coefficient and g_0 the minimum g_s (mol m^{-2} s^{-1}). The reader should be aware that this instance of the Ball-Berry model is stand-alone, and not linked to soil moisture through a land model.

The Community Land Model version 4 (CLM4)

The Community Land Model version 4 (CLM4), released in 2010 (Lawrence *et al.* 2011; Oleson *et al.* 2010) was used in this study. Land cover and atmospheric weather conditions serve as boundary conditions for CLM4. Grid cells in CLM4 may include vegetation, wetlands, lakes, glacier, and urban regions. CLM4 can be used in conjunction with the other models in the Community Earth System Model (CESM), or independently (stand-alone), as is the case here. This is referred to as an I-compset. Specifically we have used the I-compset with an f19g16 resolution and CLM4 satellite phenology. This simulation has the carbon and nitrogen cycling (biogeophysics “CN”) turned off. CLM4 parameterizes stomatal responses via a Farquhar-Ball-Berry scheme as described above.

CLM4 uses atmospheric boundary conditions for integration. We use the QIAN atmospheric input data set, for 1972-2004 (Qian *et al.* 2006). This is a global forcing dataset for the period 1948–2004 with 3-hourly temporal and T62 spatial resolution (1.875°). The dataset was developed by combining analyses of monthly precipitation and surface air temperature with intra-monthly variations from the National Centers for Environmental Prediction – National Center for Atmospheric Research (NCEP–NCAR) reanalysis (Qian *et al.* 2006). Using the I-compset we performed experiments at 350ppm, 400ppm and 700ppm. Results are provided as climatological mean values over the forcing period (1974 – 2004). Atmospheric forcing, as per Qian *et al.* (2006), is identical between each of the 350ppm, 400ppm, and 700ppm runs.

RESULTS

Free Air Carbon Dioxide Enrichment Studies (FACE)

To investigate the range of responses of g_s across global sites (Fig. 1) we performed a synthesis of data from 51 FACE studies. Of the 1313 independent measurements across 52 species, 88.2% of the measurements showed a decrease in g_s in response to elevated CO_2 (Fig. 2). However, 11.8% of the measurements showed an increase in g_s (Fig. 2). Such increases have gone largely unreported in the past, with most meta-analyses focusing on the overall mean negative response (decrease) of g_s to increasing CO_2 concentration e.g. Ainsworth and Rogers (2007). Overall, g_s decreased by ~19% on average across all FACE studies (Fig. 2).

Field survey of g_s responses to a 50ppm CO_2 rise

A total of 51 C3 tree and shrub species (n = 209) were sampled during the *in situ* CO_2 gas exchange measurements across four biomes (Fig. 3). Measurements reveal significant variation in the dynamic g_s responses to a ~50ppm CO_2 increase, which was selected to represent anthropogenic climate change over the past 25 years (from 354 to 400 ppm) across the different biomes (Fig. 3). The species of the boreal, temperate deciduous forest and tropical seasonal forest (moist) biomes displayed an overall negligible response to increasing CO_2 (Fig. 3). In contrast, the species of the tropical seasonal forest (dry) and, to an even greater extent, the species of the tropical seasonal forest (drought), which had been subjected to a one month long drought period prior to the measurements, displayed statistically significant mean increases in g_s in response to a 50 ppm rise in CO_2 (6.8% and 11.1% respectively) (Fig 3). The grouping of stomatal responses between wet (i.e. boreal forest, temperate deciduous forest, and tropical seasonal forest [moist]), and dry regions (i.e. tropical seasonal forest [dry] and tropical moist seasonal forest [drought]) is also clearly reflected in the corresponding changes in plant transpiration; decreasing and increasing mean transpiration are observed respectively (Fig. 3).

Field g_s data – model comparison

Our finding that g_s can respond positively to increasing CO_2 is supported by the theoretical predictions of the combined Farquhar-Ball-Berry (FBB) photosynthesis and g_s model. The model simulations, under a ~50ppm CO_2 rise scenario, demonstrate that increases in atmospheric CO_2 drive increases in g_s (Fig. 4) under conditions of high vapour pressure deficit [VPD] (expressed as e_a/e_i in the model) and medium-high leaf temperature (T_v). The dependence of g_s responses to increasing CO_2 on air moisture and leaf temperature is also observed in the field gas analysis data by positive correlations between g_s responses and VPD and leaf temperature (Fig. 5). This was also confirmed using mixed effects models, which showed that the measured relative changes in g_s are best explained when the relative changes in A and e_a/e_i are used as fixed factors (AIC= 1633.8 Chisq= 4.0348, p= 0.044). The FBB simulations provide a theoretical underpinning for the field observations by demonstrating that plants can increase g_s as a response to increasing CO_2, while simultaneously optimising WUE (Fig 4). In the model, increases in WUE are observed across all values of T_v and humidity. However, increases in WUE are highest in the parameter space where leaf humidity is low (dry regions) and T_v is high (warm-hot regions). A second simulation shows that the model produces an even higher g_s increase in response to a doubling of CO_2 (to 700ppm) in dry and warm-hot regions of the parameter space (not shown).

To test how well the field Infrared-Gas-Analyser (IRGA) measured g_s is described by the FBB model, as well as the optimal g_s model of Medlyn *et al.* (2011), we used the recorded values of photosynthesis (A), T_v and water vapour concentration to calculate the model-implemented g_s of all 51 taxa analyzed. For the Medlyn *et al.* (2011) model we used published g_l values by Lin *et al.* (2015) for evergreen and deciduous trees and shrubs. Here g_0 values of 20 mmol m^{-2} s^{-1} are used. The comparison of modelled and recorded data revealed that the FBB model can accurately predict the observed g_s, with the regression between estimated and observed g_s falling very close to the 1:1 line (Fig. 6). Furthermore, the model-implemented g_s responses are strikingly similar to those observed in the field (Fig. 3). A similar good fit was found when observed g_s values were plotted against the optimal g_s model of Medlyn *et al.* (2011) (Fig. S1).

The Community Land Model – a spatial investigation of global g_s

To gain a deeper understanding of the land-vegetation-system response to increases in CO_2 at a spatial global scale, we performed simulations using the CLM4 land-vegetation model. The FBB model is also used for the parameterisation of CLM4. Simulations of the same CO_2 increases in CLM4 resulted in a similar pattern of g_s responses (Fig. 7). In response to a 50ppm CO_2 increase the CLM4 simulation produces predominantly negative changes (decreases) in g_s (Fig. 7). A ~3.2% annual global climatological maximum decrease in g_s is simulated (Table 1). However, positive g_s responses are also simulated, with a maximum increase of ~4.9% (Fig. 7, Table 1). A second annual global simulation, forcing the system with a doubling of CO_2 (to 700ppm), resulted in a larger ~16.8% global climatological maximum decrease in g_s (Fig. 7). As in the 50ppm scenario, positive g_s responses were also simulated across the low latitudes, this time with higher maximum positive changes of ~18.9% (Fig. 7, Table 1). There was a clear seasonal latitudinal and regional trend in the magnitude of g_s change between months in the simulation (Fig. S2). For example, positive g_s increases (to 50ppm) were mostly observed in the months between December to May in Central Africa and June to October in South Africa. In contrast, positive g_s increases in Central America were observed in the months between January to June and in South America between June to November. Interestingly, the g_s increases were accompanied by increases in soil moisture (Fig. 8, Table 1). Annual modelled regions experiencing the increasing g_s response to CO_2 include countries such as Mexico, the Galapagos Islands, Dominican Republic, Columbia, Venezuela, Brazil, Bolivia, Sudan, South Sudan, Somalia, Tanzania, Democratic Republic of Congo (D.R.C.), Angola, Namibia, Botswana and Indonesia (Fig. 7, Table 2). Similar to our field observations, areas that showed positive g_s increases were situated in hot and dry biomes (Table 2).

DISCUSSION

Overall, our results clearly demonstrate that in dry, warm environments, or during drought periods, plants can respond to increases in CO_2 by increasing their g_s, while, crucially, maximising the increase in their WUE (Figs. 3, 4, 7) compared to plants growing in the cooler moist conditions of the temperate latitudes. Implementation of the FBB model clearly shows a region of parameter space where CO_2, g_s and WUE increases can coincide (Fig. 4).

The FBB model, when supplied with independently-measured values of V_{cmax}, was able to accurately predict field observations, including the unexpected increases in g_s at high T_v and high VPD (Figs. 3, 6), a region of parameter space not often explored in standard gas analysis protocols which typically run under standardized temperatures and VPD of 22˚C and 1kPa respectively. Although the measured g_s responses are small and difficult to capture under field conditions, Figs. 3 and 6 show excellent agreement between modelled and observed values and strongly support our claims.

For a more mechanistic understanding of the g_s responses documented above, we turn to a more detailed analysis of the FBB model. Firstly, we note that in the light-saturated conditions we are exploring here, A is Rubisco-limited and is thus expected to increase with temperature. In the particular formulation used here (see Materials and Methods), V_{cmax} increases roughly exponentially with temperature at temperatures below ~35°C, leading to a strong steepening of the A-c_i response curve as temperature increases (Fig. 4). This steepening carries over to the A-c_a response, as shown in Fig. 4; this figure also shows that higher humidity yields greater A at a given temperature and c_a, because greater humidity promotes stomatal opening (Fig. 4) and thus greater c_i, enhancing photosynthesis. Furthermore, we note that Equation (1) in the model (see Materials and Methods) implies that the sensitivity of g_s to ambient CO_2, dg_s/dc_a, at fixed temperature and humidity is given by:

$$\frac{c_a^2 e_a}{mAp_a e_i}\frac{dg_s}{dc_a} = \frac{c_a}{A}\frac{dA}{dc_a} - 1 \qquad (5)$$

Thus, increasing g_s in response to increasing c_a is possible when the first term on the right-hand side is greater than one, i.e. when the relative change in A is greater than the relative change in c_a. This condition can be met when temperature is high and humidity is low (as exemplified by the solid circles in Fig. 4): in that case, dA/dc_a is high while A is low, bringing dg_s/dc_a above zero (Fig. 4). When both temperature and humidity are high (squares in Fig. 4), A is large enough to make the first term on the right less than one; conversely, when both temperature and humidity are low (triangles in Fig. 4), A is low but dA/dc_a is also low, and the first term on the right is still less than one.

In summary, the FBB model predicts $dg_s/dc_a > 0$ at high temperature and low humidity under light-saturated conditions because high temperature promotes a strong gain in A per unit increase in c_i (or c_a), while low humidity keeps the base value of A low. Naturally, different model formulations would give quantitatively different results; in particular, the threshold values of temperature and humidity required for $dg_s/dc_a > 0$ are likely to be strongly model-dependent. However, the qualitative nature of the result appears robust, since increasing V_{cmax} with increasing temperatures and stomatal opening with increasing humidity are both well-known features of plant physiology. Note in particular that the optimization models of Medlyn *et al.* (2011) also predict increasing g_s as humidity increases (or VPD decreases), and would thus give qualitatively similar behaviour to the empirical Ball-Berry closure reported here (Fig. S1).

It is surprising that the possibility of g_s increases as a response to rising CO_2 under these particular climatic conditions has not been highlighted before. As implied above, optimization models also predict similar increases within the CO_2 envelope tested in the present study (i.e. 354-400ppm CO_2) (Arneth *et al.* 2002; Konrad *et al.* 2008; Medlyn *et al.* 2013; Medlyn *et al.* 2011). For example, the optimization model of Konrad et al. (2008) demonstrates that the inflection point between rising and falling g_s response to CO_2 is dependent on the 'cost of water' (Fig. 4 in their article). In particular, high cost of water shifts the inflection point to higher values, which are similar to those used in the present study. These predictions fit well with both our measured and modelled g_s responses.

It is intriguing that a substantial number of the FACE studies (see Materials and Methods) also report increases in g_s under super-ambient CO_2. These increases in g_s are mostly not discussed, or are disregarded as methodological artefacts (Gunderson *et al.* 2002). Due to a lack of standardised FACE protocols, the exact reasons why positive g_s responses are observed across these studies remain largely unclear. Possible reasons for the observed increases might include; a) differences in the climatic and/or cuvette measurement conditions; b) differences in soil nutrient and water status; c) differences in the signal to noise ratio with regard to g_s (i.e. species with low g_s show a greater propensity for erroneous measurements); d) studies do not consistently record the time when measurements are taken, despite literature which shows that g_s responses to CO_2 are highly dependent on the time of day (Konrad *et al.* (2008). Unfortunately, FACE studies inherently include a range of weather regimes/cuvette conditions and measurement times, which are inconsistent amongst studies and typically unreported. It is therefore not possible to assess the role of these conditions with regard to the reported g_s increases. Secondly, nutrient concentrations and soil water content naturally vary between sites, but are inconsistently documented across studies [e.g. Naumburg *et al.* (2003)] making direct comparison unfeasible at this time. Regarding the potential low signal to noise ratio of the species that display increases in g_s as a response to increased CO_2, our meta-analysis of FACE studies showed that there is no significant difference in the g_s values between species that show either positive or negative responses to CO_2 ($F=1.663$, $p=0.198$). The same was found to be the case for the g_s responses of different PFTs, with the exception of shrubs ($F=4.122$, $p<0.001$). Thus, the observed positive g_s responses in FACE studies may arise for a number of different reasons. If we were to speculate, it is likely that at least part of them are due to warm, dry conditions, as demonstrated by our field data (Fig. 3, 5) and model comparisons (Fig. 6 and Fig. S1).

Positive g_s responses have the potential to alter regional or even global hydrological and carbon cycles, and other ecological processes. We acknowledge that there are limitations in assessing long term g_s trends through field measurements, as they cannot account for long term water availability changes resulting from the CO_2 effects on g_s. Several studies have shown that decreasing soil moisture can elicit greater stomatal closure under elevated CO_2 than ambient CO_2 (Leakey et al., 2006; Piao et al., 2007; Gray at al., 2016). Similarly, increases in LAI has been shown to reduce soil moisture, thus indirectly affecting g_s (Field *et al.* 1995; Wenfang *et al.* 2013). Our global simulations using CLM can only partially test for this, as LAI was not simulated here. It also needs to be noted that current CLM parameterizations do not account for many morphological plant responses to elevated CO_2 (e.g. changes in stomatal density). Keeping these reservations in mind and although predictions of future g_s are somewhat beyond the scope of the present study, Fig. 8 shows that in regions where g_s is predicted to increase in response to a 50 and 350ppm CO_2 rise, soil moisture also increases (in this instance the increased soil moisture may be caused by water savings due to suppressed g_s in prior months, and may in fact cause the annual mean increase of $g_{s.}$ at these locations). Coupled with potential increases in LAI in response to elevated CO_2 (Frank *et al.* 2015; Niu *et al.* 2013; Piao *et al.* 2007; Schymanski *et al.* 2015; Wu *et al.* 2012), regionally increasing g_s may act to offset the much studied effects of decreasing g_s e.g. increasing river runoff (Betts *et al.* 2007; de Boer *et al.* 2011; Gedney *et al.* 2006; Gopalakrishnan *et al.* 2011; Lammertsma *et al.* 2011), or even drive enhanced drought and desertification in certain regions (Dai 2013). Areas that were predicted by CLM to show increases in g_s with elevated CO_2 (~50 and 350ppm) are located in hot and dry biomes (Fig. 7 and Table 2). A monthly analysis of g_s for CLM also suggests that the relative timing of temperature and relative humidity is important in driving the g_s increases; which leads us to expect increases in g_s in monsoonal regions (Fig. S2). However, due to other confounding factors (e.g. vegetation types and/or soil moisture) this expectation is not always met (e.g. India) and requires further investigation which is beyond the scope of the current study. Continued land-vegetation model development based on field data at the biome (and community-species) level, as well as further Earth System Model inter-comparison studies, will be required to assess the implications of this shift in our understanding of vegetation responses to elevated CO_2, and for improved prediction of the global hydrological cycle, particularly in dry and warm-hot regions.

We demonstrated that increases in g_s can occur under elevated CO_2 in environments that are hot and dry (high VPD). Our field observations across several global biomes are in excellent agreement with predictions from optimization models and fall within a previously unrecognised parameter space within the FBB model. The implications of our findings are of global significance for future modelling of soil-vegetation-climate feedbacks, as the FBB model is also implemented in CLM. Although the majority of the global vegetation respond by decreasing g_s under elevated CO_2, biomes that already experience drought conditions are likely to show increases in g_s. It remains to be seen how these increases will affect soil-canopy-atmosphere climate feedbacks in the future, particularly in areas that are already expected to be more threatened as a result of predicted changes in climate.

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SUPPLEMENTAL MATERIAL (see separate files)

Table S1. Species list and site description

Figure S1. Comparison of measured and modelled g_s values under 354 and 400ppm of atmospheric CO_2 using the optimal g_s model by Medlyn *et al.* (2011).

Figure S2. Stomatal conductance response to increasing CO_2 in the CLM4 land-vegetation model for each month of the year. Negative and positive g_s responses to increasing CO_2 in CLM4 (400ppm relative to 350ppm).

FIGURE LEGENDS

Figure 1. The location of FACE studies included in our assessment. 51 FACE studies are shown (most overlap on this scale). Most FACE studies are located in Northern hemisphere locations between 30-60° North. FACE studies which did not, to our knowledge, document g_s changes were not included. See Materials and Methods for all cited studies used.

Figure 2. FACE synthesis of g_s responses to increasing CO_2 concentration. Kernel density probability distribution of the percentage change of g_s to increasing CO_2 concentration. Each colour represents a different Plant Functional Type (PFT). The percentage g_s change is expressed as the delta change of g_s between ambient and high CO_2 treatments. Solid lines are median (blue) and mean (red) change in g_s across all PFTs. The dashed line is the zero percentage change mark. See Materials and Methods for details of the synthesis and cited FACE studies used.

Figure 3. Dynamic g_s responses to a subtle CO_2 increase across four biomes observed in field conditions compared with modelled responses. A, Percentage change in g_s during the transition from 354 (sub-ambient) to 400ppm (modern ambient) atmospheric CO_2, which is representative of atmospheric changes that have occurred over the past ~25 years. The boxes signify the distribution of the 25%–75% quartiles, with median and average values represented by a vertical line and an open square within the box, respectively. The whiskers indicate the distribution of the 5–95% quartiles. Solid boxes represent the field

measurements. Stripped boxes represent the modelled percentage responses of g_s using the Farquhar-Ball-Berry model and the A, T_v and e_a/e_i values measured in the field. Different letters denote statistically significant differences between biomes ($p \leq 0.05$). Asterisks indicate within-biome statistically significant differences between the conductance values at 354 and 400ppm of CO_2. N= 24-66 independent measurements depending on biome (see Table S1 for species list). B, Percentage change in transpiration between 354 and 400ppm atmospheric CO_2. C, Locations of expedition sites visited during this study. See Table S1 for geographical coordinates and site information.

Figure 4. Results from the Farquhar-Ball-Berry combined photosynthesis and g_s model. A, A-c_i response curves at two different leaf temperatures, as indicated in the legend. B, A-c_a response curves at two different temperatures and humidities (see legend in panel C). C, Sensitivity of A to c_a, normalized by A/c_a, as a function of c_a at two different temperatures and humidities, as indicated by the legend. D, Predicted g_s at c_a = 350ppm as a function of leaf temperature and humidity. E, Predicted percentage change in g_s when c_a changes from 350 to 400ppm, with zero contour highlighted by solid black line. F, Predicted percentage change in water use efficiency WUE when c_a changes from 350 to 400ppm. Symbols in all panels indicate three selected cases: high temperature, low humidity (circles); high temperature, high humidity (squares), and low temperature, low humidity (triangles).

Figure 5. Gas analysis relationship between g_s and vapour pressure deficit and leaf temperature. Linear relationship and 95% confidence bands (dotted lines) between the percentage change in g_s during the transition from 354 (sub-ambient) to 400ppm (modern ambient) atmospheric CO_2 and A, VPD (kPa) (y=5.94x-5.24, r^2=0.21, p<0.01) and B, leaf temperature (°C) (y=0.63x-12.82, r^2=0.14, p<0.01). Data represent species averages with an

average number of four individuals measured per species.

Figure 6. Comparison of measured and modelled g_s values under 354 and 400ppm of atmospheric CO_2. Relationship (0.95x+6.8, r^2=0.431, solid line) between measured and modelled g_s values. Stomatal conductance was modelled using the Farquhar-Ball-Berry model and the A, T_v and e_a/e_i values measured in the field. The dashed line represents the 1:1 relationship. Mixed effects model results showed that the relative changes in g_s are best explained when the relative changes in A and e_a/e_i are used as fixed factors (AIC= 1633.8 Chisq= 4.0348, p= 0.044).

Figure 7. Annual g_s response to increasing CO_2 in the CLM4 land-vegetation model. Negative and positive g_s responses to increasing CO_2 in CLM4, for A, a 400ppm and B, a 700ppm scenario, relative to 350ppm. Modelled regions experiencing positive g_s responses for both A, and B, include parts of Central America, South America, Africa and Asia (see Table 2 for more detail). It should be noted that the majority of the land surface experiences decreases in g_s in response to increasing CO_2.

Figure 8. Detailed analysis of CLM grid cells showing positive g_s responses under a 400 and 700ppm CO_2 scenario. Percentage change of soil moisture and g_s for a 400ppm (solid lines) and a 700ppm (dashed lines) scenario, relative to 350ppm. Only grid cells that showed positive increases in g_s are used for this analysis (geographical areas coloured in reds and oranges in Fig. 7).

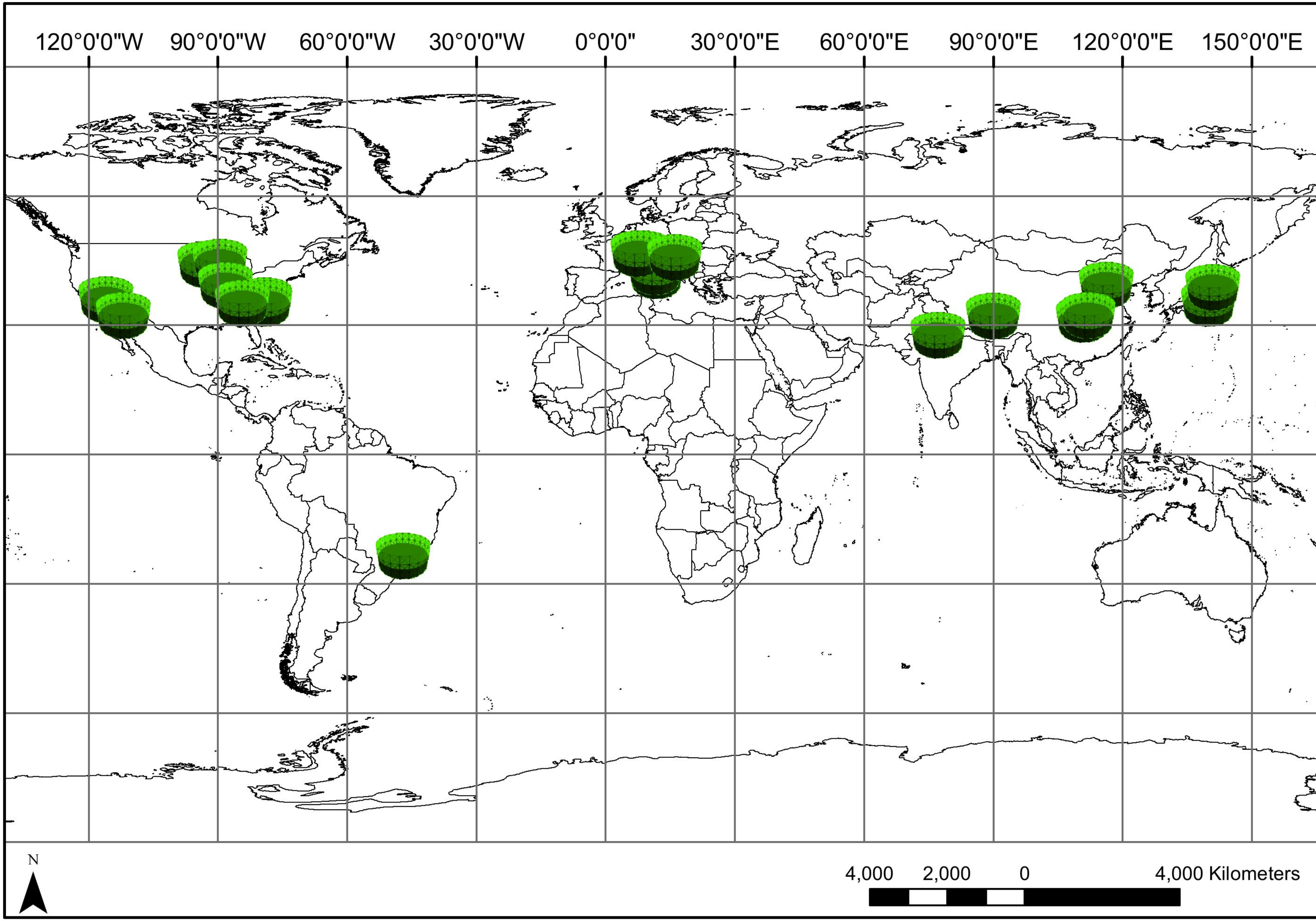
Table 1. CLM maximum annual increases/decreases and percentage of grid cells showing increases/decreases or no change in g_s and soil moisture across the globe.

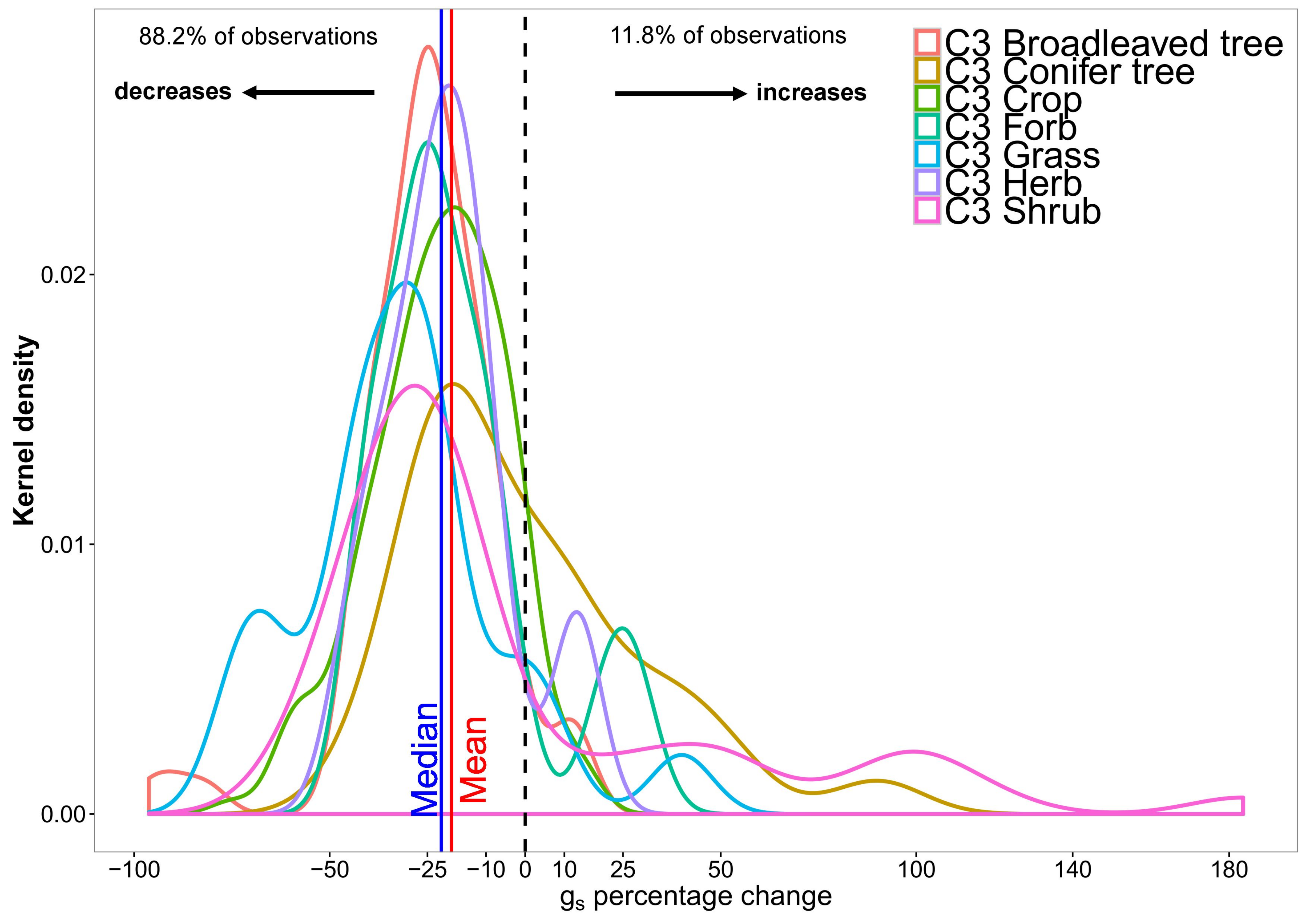
CO_2 (ppm)	**Variable**	**Max. decreases**	**Max. increases**	**Percentage no. of grid cells**		
				Increase	Decrease	No change
400-350	Stomatal conductance (s/m)	0.00075 (3.15%)	0.00004 (4.92%)	1.94	64.22	33.83
	Soil moisture (kg/m^2)	0.1 (0.21%)	1.1 (2.3%)	48.55	0.15	51.33
700-350	Stomatal conductance (s/m)	0.00004 (16.82%)	0.00001 (18.94%)	1.45	65.81	32.74
	Soil moisture (kg/m^2)	2.6 (5.6%)	0.01 (0.02%)	80.87	0.03	19.11

Table 2. Countries and associated biomes that showed an annual positive increases in g_s under a 50ppm increase in CO_2.

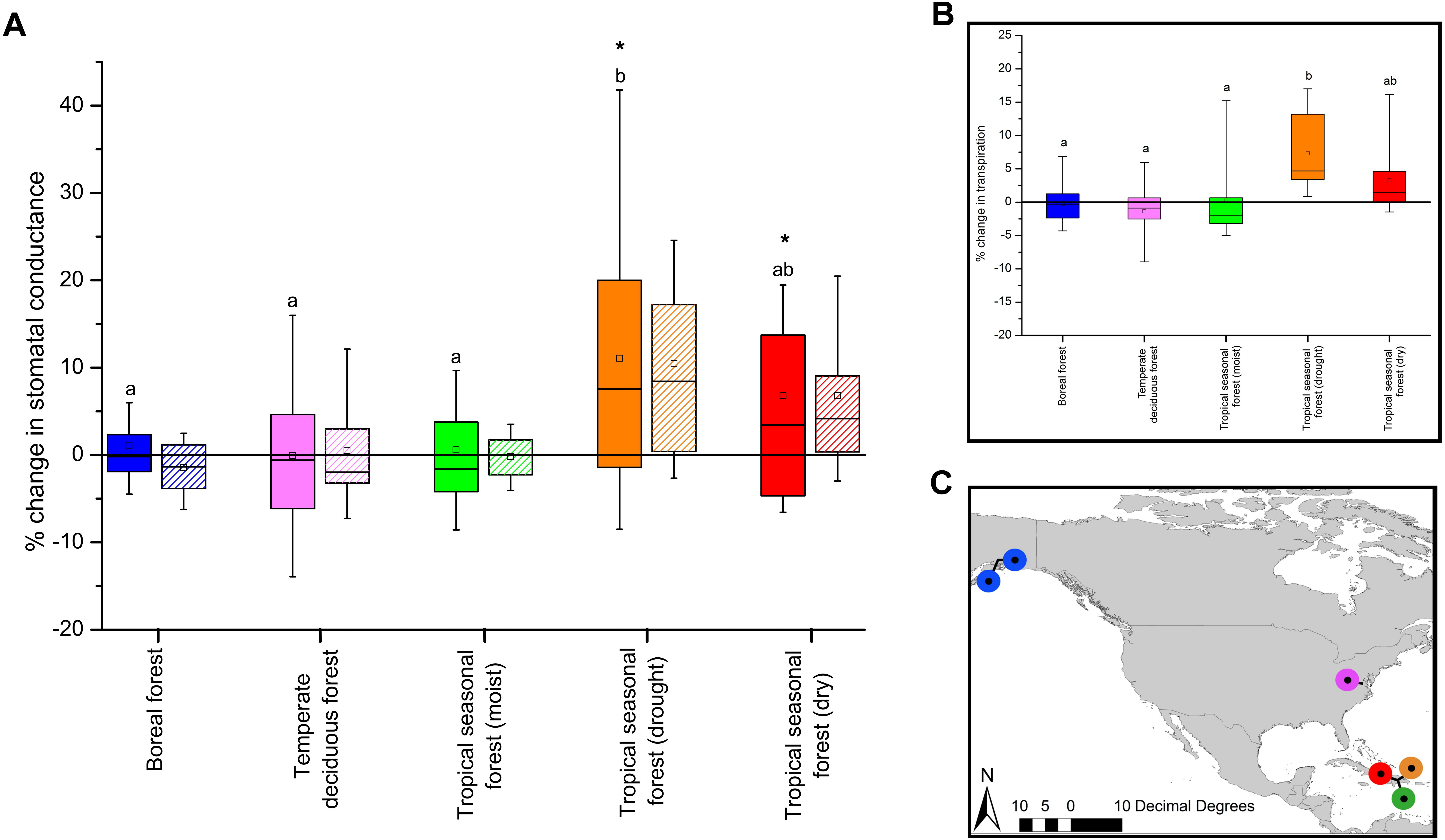
Continent	**Country**	**Biome**
Central America	Mexico	Tropical & Subtropical Dry Broadleaved Forest
South America	Galapagos Island	Mediterranean Forests, Woodland & Shrub
South America	Dominican Republic	Tropical & Subtropical Dry Broadleaved Forest
South America	Columbia	Tropical & Subtropical Dry Broadleaved Forest & Deserts & Xeric Shrublands
South America	Venezuela	Deserts & Xeric Shrublands
South America	Brazil	Deserts & Xeric Shrublands
South America	Bolivia	Tropical & Subtropical Grasslands, Savannas & Shrublands
Africa	Sudan	Tropical & Subtropical Grasslands, Savannas & Shrublands
Africa	South Sudan	Tropical & Subtropical Grasslands, Savannas & Shrublands
Africa	Somalia	Tropical & Subtropical Grasslands, Savannas &

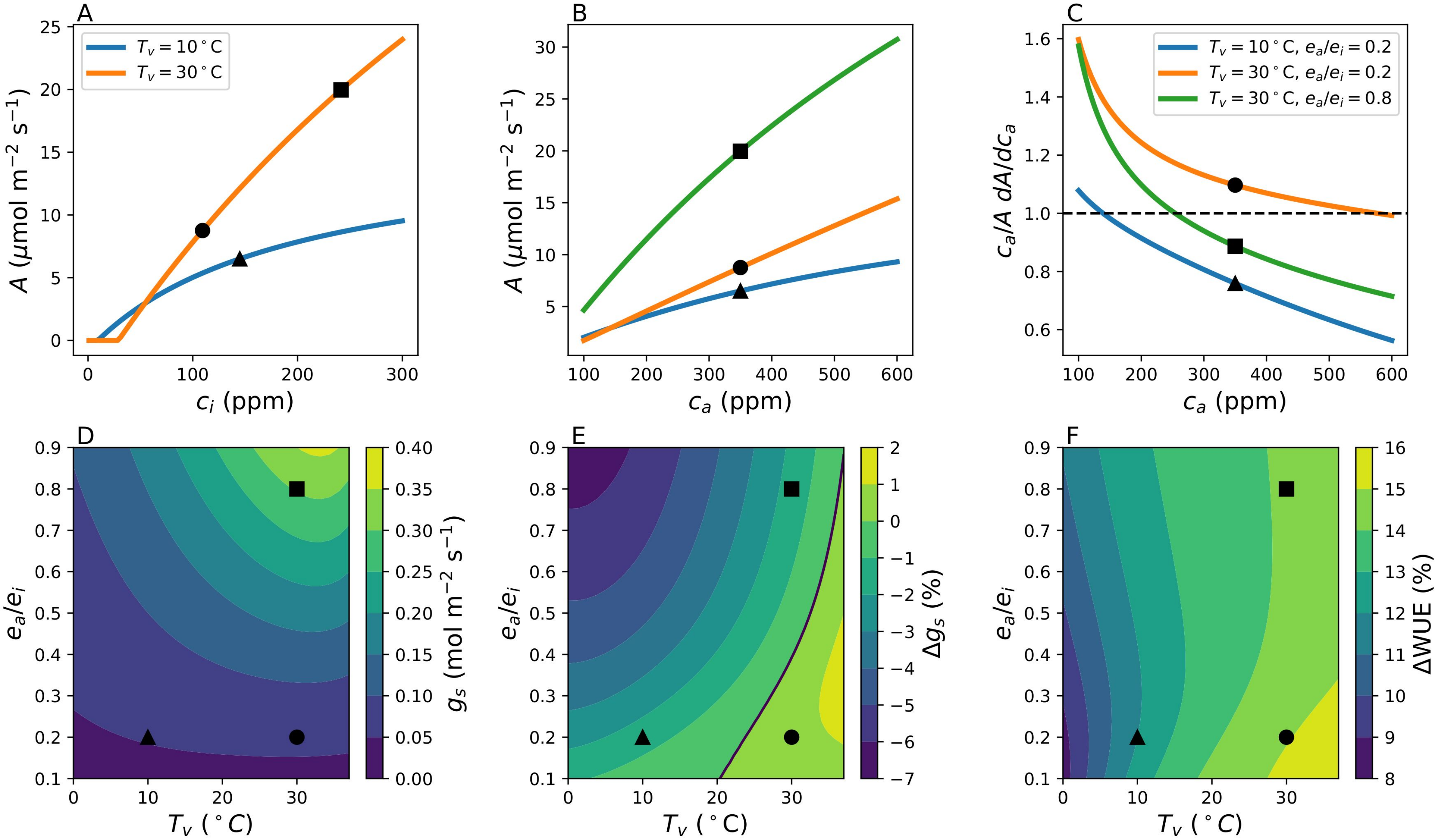
		Shrublands
Africa	Tanzania	Tropical & Subtropical Grasslands, Savannas & Shrublands
Africa	D.R.C.	Tropical & Subtropical Grasslands, Savannas & Shrublands
Africa	Angola	Tropical & Subtropical Grasslands, Savannas & Shrublands
Africa	Namibia	Tropical & Subtropical Grasslands, Savannas & Shrublands
Africa	Botswana	Tropical & Subtropical Grasslands, Savannas & Shrublands
Asia	Indonesia	Tropical & Subtropical Dry Broadleaved Forest


120°0'0"W
90°0'0"W
60°0'0"W
30°0'0"W
0°0'0"
30°0'0"E
60°0'0"E
90°0'0"E
120°0'0"E
150°0'0"E
90°0'0"
60°0'0"N
30°0'0"N
0°0'0"
30°0'0"S
60°0'0"S
90°0'0"
N
4,000
2,000
0
4,000 Kilometers

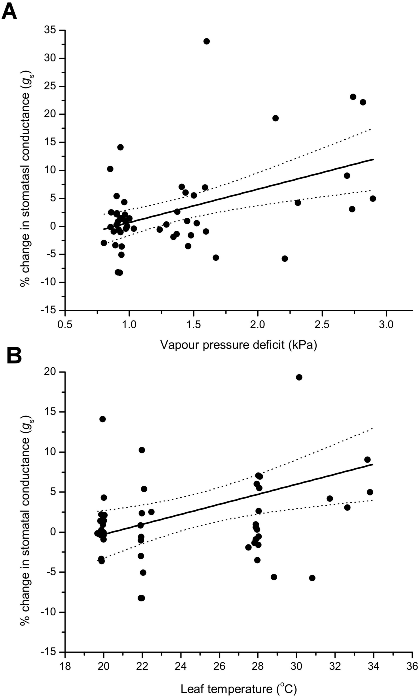


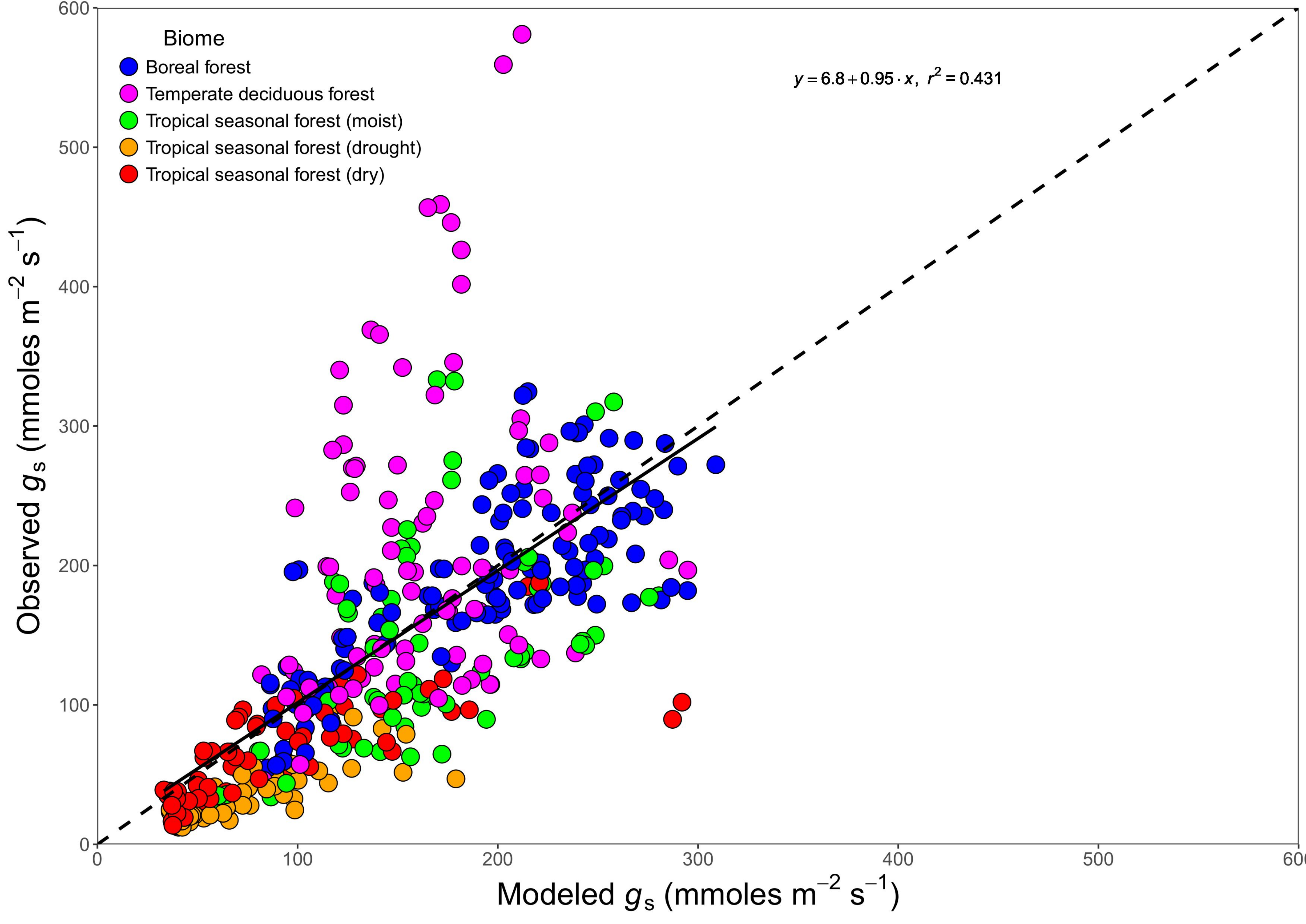
88.2% of observations
11.8% of observations
decreases
increases
C3 Broadleaved tree
C3 Conifer tree
C3 Crop
C3 Forb
C3 Grass
C3 Herb
C3 Shrub
Kernel density
0.00
0.01
0.02
Median
Mean
−100
−50
−25
−10
0
10
25
50
100
140
180
gs percentage change


A
% change in stomatal conductance
40
30
20
10
0
-10
-20
a
a
a
*
b
*
ab
Boreal forest
Temperate deciduous forest
Tropical seasonal forest (moist)
Tropical seasonal forest (drought)
Tropical seasonal forest (dry)
B
% change in transpiration
25
20
15
10
5
0
-5
-10
-15
-20
a
a
a
b
ab
Boreal forest
Temperate deciduous forest
Tropical seasonal forest (moist)
Tropical seasonal forest (drought)
Tropical seasonal forest (dry)
C
N
10 5 0
10 Decimal Degrees

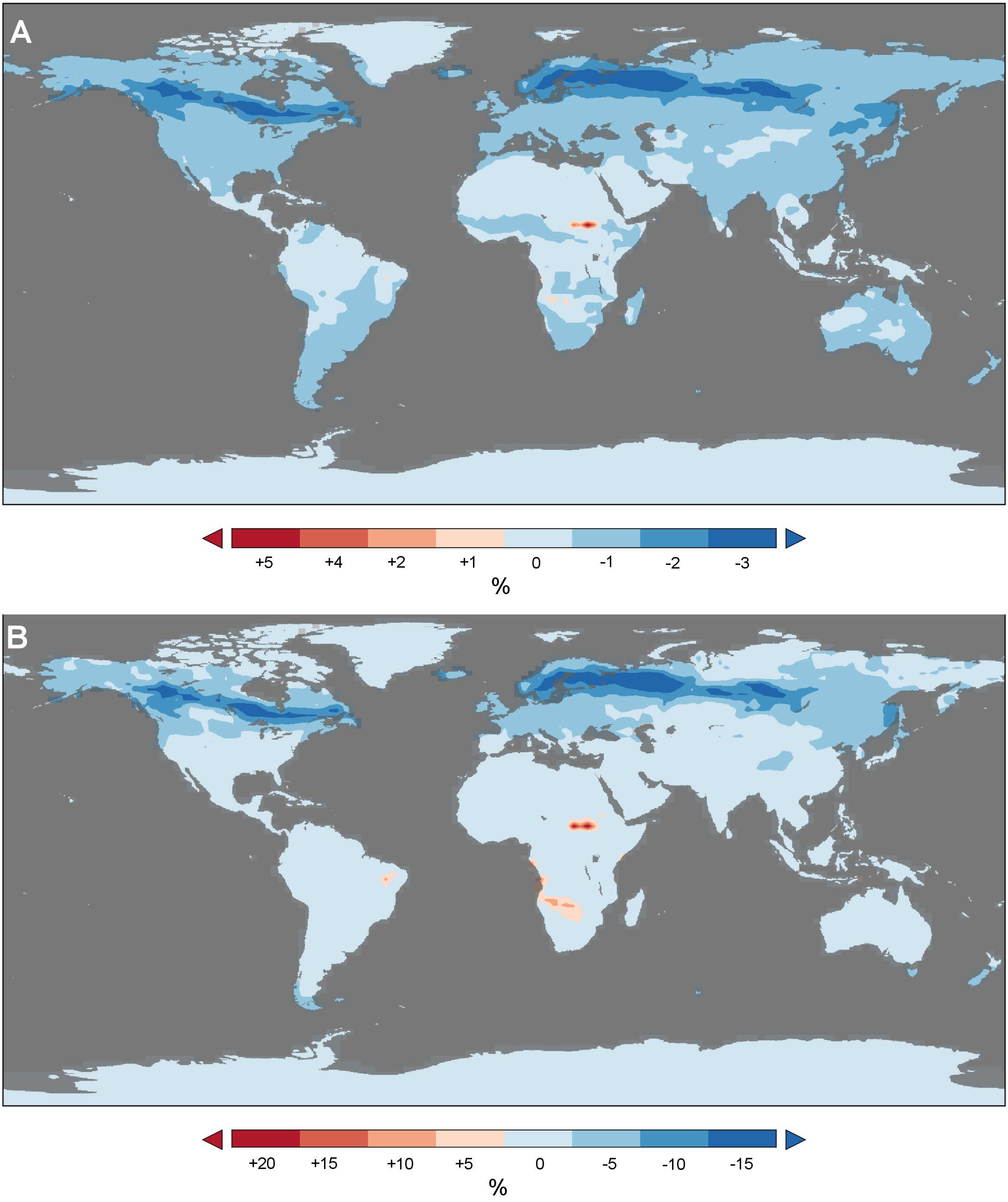


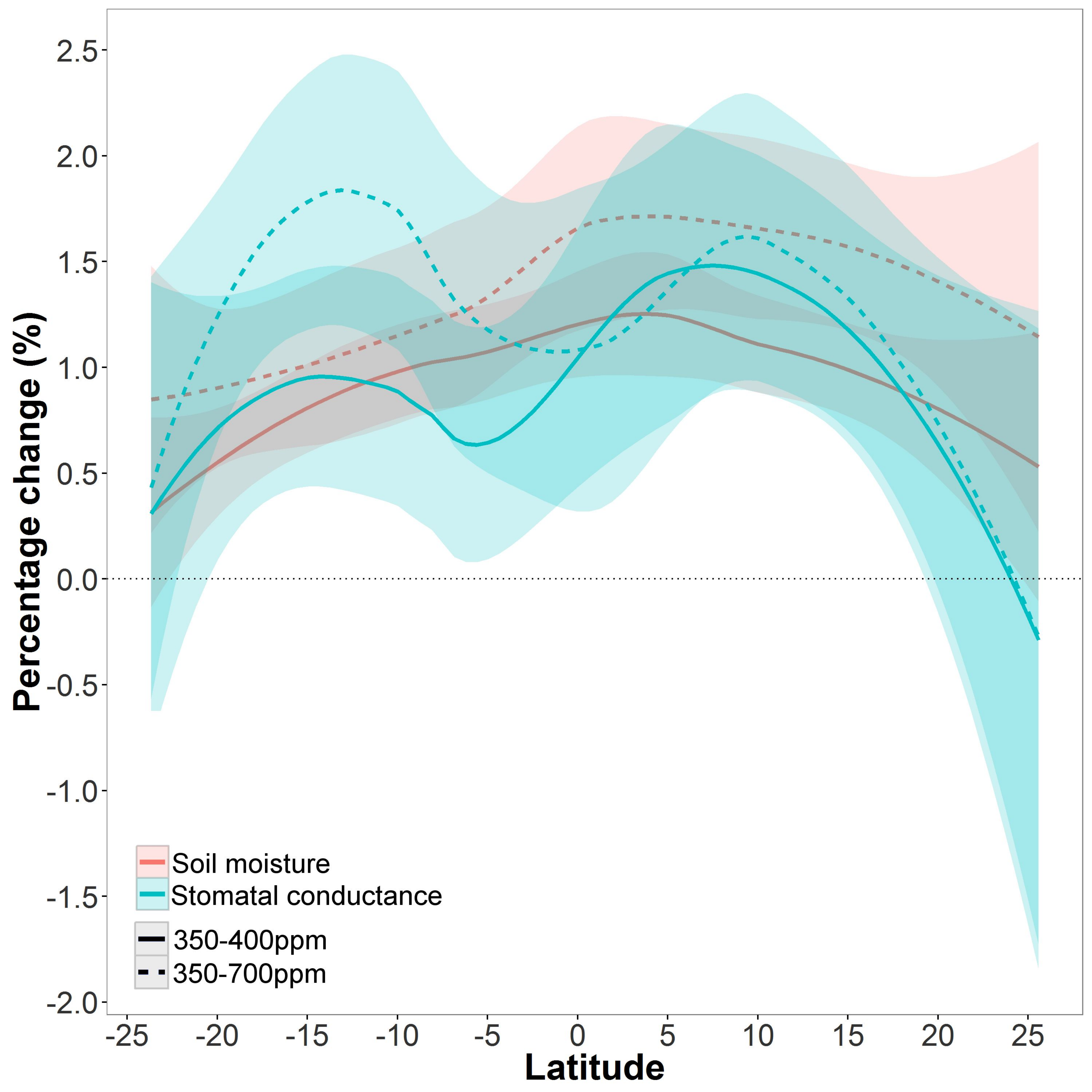
A
B
C
D
E
F
$T_v = 10^\circ$C
$T_v = 30^\circ$C
$T_v = 10^\circ$C, $e_a/e_i = 0.2$
$T_v = 30^\circ$C, $e_a/e_i = 0.2$
$T_v = 30^\circ$C, $e_a/e_i = 0.8$
A (μmol m^{-2} s^{-1})
c_i (ppm)
c_a (ppm)
$c_a/A\ dA/dc_a$
e_a/e_i
T_v ($^\circ C$)
g_s (mol m^{-2} s^{-1})
Δg_s (%)
ΔWUE (%)


A
% change in stomatasl conductance (g_s)
Vapour pressure deficit (kPa)
35
30
25
20
15
10
5
0
-5
-10
-15
0.5
1.0
1.5
2.0
2.5
3.0
B
% change in stomatal conductance (g_s)
Leaf temperature (^{o}C)
20
15
10
5
0
-5
-10
-15
18
20
22
24
26
28
30
32
34
36



Biome
Boreal forest
Temperate deciduous forest
Tropical seasonal forest (moist)
Tropical seasonal forest (drought)
Tropical seasonal forest (dry)
$y = 6.8 + 0.95 \cdot x, \; r^2 = 0.431$
Observed g_s (mmoles m^{-2} s^{-1})
Modeled g_s (mmoles m^{-2} s^{-1})
0
100
200
300
400
500
600


A
+5
+4
+2
+1
0
-1
-2
-3
%
B
+20
+15
+10
+5
0
-5
-10
-15
%



Percentage change (%)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-25
-20
-15
-10
-5
0
5
10
15
20
25
Latitude
Soil moisture
Stomatal conductance
350-400ppm
350-700ppm